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OFFICE OF NAVAL RESEARCH
Microbiology Branch

SEMI-ANNUAL PROGRESS REPORT

Report Prepared By: C. Phillip Miller Date: 25 July, 1952

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CONTRACTOR: University of Chicago

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TITLE OF PROJECT: The Effect of Antibiotics on Bacteria

Objectives: To determine the effect of antibiotics on the intestinal flora of mice subjected to total body x-radiation, with special attention to those enteric micro-organisms which have been found to be particularly important in causing fatal bacteremia after exposure to 550 r total body x-radiation.

SUMMARY OF RESULTS:

1. This report is longer than usual because it describes a number of uncompleted experiments of a preliminary nature which have been made in an attempt to explore several avenues of approach for the most promising lead to the solution of a complicated problem.

2. In an attempt to gather information about the time after total body irradiation when potentially pathogenic bacteria in the intestinal tract penetrate the mucosa and initiate systemic infection, the following experiment was carried out: On different days post-irradiation, the mice were fed different strains of Pseudomonas aeruginosa, each of which had been made resistant to a

different antibiotic. This procedure made it possible to identify the strains as they were recovered from heart's blood at autopsy and thereby to determine the time at which each particular strain had been administered. The bacteria were given by mixing them with the drinking water. The sequence of the different strains was changed in different experiments because the chloromycetin-resistant strain seemed to be less virulent than the others. The mice were irradiated with 550 r.

The strain which was fed during the first two days after irradiation caused a higher incidence of fatal bacteremia than those fed later on.

3. To determine the infective dose of Pseudomonas by mouth in an irradiated animal, a streptomycin-resistant strain was fed to irradiated mice during the first 24 hours post-irradiation and in another experiment on the sixth day post-irradiation. A streptomycin-resistant strain was used in order to identify it easily. Blood cultures were made on all the mice which died. The following table gives the percentage of mice showing this strain in their blood cultures. It shows that a smaller inoculum was required on the sixth day post-irradiation than during the 24 hours immediately following irradiation.

TABLE I. PERCENTAGE OF AUTOPSY BLOOD CULTURES CONTAINING DRUG-RESISTANT PSEUDOMONAS

	Approx. No. Pseudomonas per ml. in Drinking Water					
	10^8	10^7	10^6	10^5	10^4	10^3
Fed on day of irradiation	62 %	33 %	0 %	0	10 %	
Fed on 6th day post-irradiation	100	87	31	0	0	0

A polymyxin-sensitive strain of Pseudomonas was fed to mice irradiated with 550 r during the first 48 hours after irradiation. Polymyxin was then

given by mouth in their drinking water (20 µg/ml. of water) every day thereafter. In spite of this treatment all of the mice died with Pseudomonas bacteremia, and all of the strains recovered from heart's blood were found to be polymyxin-sensitive. The administration of polymyxin by mouth, therefore, failed to eliminate the Pseudomonas which had been introduced into the gastrointestinal tract.

4. A systematic survey was made to determine the incidence of Salmonella carriers among our stock mice as they were received from the breeder (Rockland Farms, New City, New York). This was necessitated by an unusually high incidence of generalized infection with Salmonella enteritidis in irradiated mice.

Groups of approximately fifty mice were examined in the following way: A sample of feces from each mouse was cultured, and each mouse isolated individually in a separate jar. Fresh fecal specimens were cultured each day thereafter for the duration of the observation period. Cultures were made in tetrathionate broth and subcultured the following day onto eosin-methylene-blue agar, SS agar, and blood agar. Particular attention was paid to Salmonella, Proteus, and Pseudomonas.

It was found that although Salmonella enteritidis was recovered from a high percentage of mice (up to 45 per cent) on arrival in the laboratory, about half of these became Salmonella-negative thereafter. Those with consistently positive fecal cultures were designated persistent carriers; those which lost Salmonella from their fecal cultures after isolation were designated transient carriers.

Mice which had been cultured in this manner for three to four weeks were poisoned with nitrogen mustard (0.2 mg intraperitoneally), and after death a few days later, were autopsied for cultures of heart's blood and spleen. Cul-

tures of the persistent carriers always contained Salmonella. Those of the transient carriers never did. Incidentally, the persistent carriers died sooner than the others.

A small epidemiological study was made in which small groups of Salmonella-negative mice were exposed to a persistent carrier by housing them in a cage together after a preliminary period of isolation. Each mouse was cultured each day for the following three weeks. During this period of contact Salmonella was recovered from the feces of only two mice, from each on only one occasion. (See Table II.)

When this experiment was repeated with mice irradiated with 550 r and then exposed to a carrier, Salmonella promptly appeared in the feces of most of the mice. This experiment is still in progress.

Similar epidemiological studies have been made with Proteus; i.e., mice which had been Proteus-negative for three weeks, or had been positive for Proteus on no more than one occasion, were put together with a persistent Proteus carrier. (See Table III.) Some of the contacts had occasional positive cultures. But when the mice were irradiated at the time of mixing, all of the contacts became positive for Proteus.

Mice which had remained Proteus-negative during 4 weeks exposure to a carrier and were then x-rayed and segregated again continued to remain Proteus-negative.

Although these observations are too few to justify any conclusion, they do suggest that these micro-organisms are able to establish themselves much more readily in the intestinal tract in irradiated mice than in normal mice during contact with an infected cage mate; i.e., by the natural mode of spread of epizootic infection. It should be pointed out that these mice come from a stock

known to contain carriers of both of these micro-organisms and have therefore been exposed to them, and consequently have a fairly high natural resistance to them. (See Section 5.)

While it is realized that epidemiological experiments carried out on such mice are more difficult to interpret than experiments made on animals known to be free of the infection under investigation, it is felt that they simulate more closely the herd conditions which might prevail in a human population suddenly exposed to mid-lethal doses of ionizing radiations. It is regrettable that the great amount of work involved severely limits the number of animals which can be studied. An attempt is being made to overcome this difficulty by simplifying the technique and thereby increasing the size of each experiment.

5. The natural resistance of these mice to Salmonella infection was demonstrated in the following experiment. From among a large number of mice kept in individual jars and cultured daily for 3 to 4 weeks, 27 which were found to be consistently Salmonella-negative were given Salmonella enteritidis by mouth. They were continued in isolation and cultured daily until death, or until they were killed for autopsy cultures. The feces of those which were fed with approximately 10,000 micro-organisms contained Salmonella only during the first 24 hours after inoculation.

Nearly half of those which received 20,000 Salmonella became negative in 2 days. Even some of those receiving 100,000 became negative. Some of the mice receiving 20,000 or 100,000 died of Salmonella infection.

6. Our preceding progress report (dated 29 January, 1952) stated that we had been unable to obtain consistent confirmation of our earlier observation that treatment with very small doses of streptomycin or terramycin increased the mortality of mice subjected to 550 or 600 r total body x-radiation. Fifteen

additional experiments of this kind have since been made. In 5 of 8 experiments with terramycin and in 3 of 7 with streptomycin, the animals died more rapidly than the untreated controls. In the other 7 experiments there was no difference between treated and untreated mice.

An apparatus has been constructed (through the courtesy of the Institute for Radiobiology and Biophysics) which will permit growth of different strains of bacteria on either side of a sintered glass filter, but will allow the culture medium to flow back and forth. All of the technical difficulties have not yet been solved, however, so that it has not been put to use.

PLANS FOR THE FUTURE

Work will be continued on:

1. The effect of antibiotics on the intestinal flora of normal and irradiated mice.

2. Epidemiological experiments with irradiated mice.

3. The effect on the relative growth rates in vitro of enteric bacteria growing in a common culture medium, but separated in a bacteria-tight filter.

REPORTS AND PUBLICATIONS

1. C. Phillip Miller - Clinical and Laboratory Aspects of Antibiotic Therapy. Lecture in the course on Military Medicine for Medical Officers, Army Medical Service, Graduate School, Washington, D. C., 15 March, 1952.

2. Carolyn W. Hammond, C. Phillip Miller, Marianne Tompkins, Margaret Colling, and Marjorie Bohnhoff - Duration of Post-Irradiation Bacteremia in Mice. Paper presented at the Annual Meeting of the Society of American Bacteriologists, Boston, Massachusetts, 28 April, 1952.

Respectfully submitted,

C. Phillip Miller

TABLE II. PRESENCE OR ABSENCE OF SALMONELLA IN REPEATED CULTURES OF FECES

Irradiated Mice

Days before and after	Isolated						Housed Together				
	17	13	10	8	4	1	1	4	8		
	- - - - -	- - -	- - -	- - +	+	550 r x-ray	+	+ + +	+D	HB: Salmonella, Coli	
	- - - - -	- - -	- - - -	-			-	- + -	+ -		
	- - - - -	- - -	- - - -	-			-	- + -	+ -		
	- - - - -	- - -	- - - -	-			-	- - -	+ -		
	- - - - -	- - -	- - - -	-			-	+ - -	- -	(Experiment still	
	- - - - -	- - -	- - - -	-			-	- - -	- -	in progress)	

Unirradiated Mice

Days	Isolated					Housed Together							
	14	10	7	4	1	1	4	7	12	15	19	23	
	- + + + +	+ + + +	+			+ + + +	+ + + +	+	+ -	- + + +	-		
	- - -	- - -	-			- - - -	- - - - -		+ -	- -	-		
	- - - - -		-			- - - -	- - - - -		- -	- - - -	-		
	- - - - -	- - -	-			- - - -	- - - - -		- -	- - - -	-		
	- - - - -	- - -	-			- - - +	- - - - -		- -	- - - -	-		
	- - -	- - -	-			- - - -	- - - - -		- -	- - - -	-		
	+ + + + +	+ + + +	-			+ + + +	+ + + +	- -	+ -	+ + + +	-		
	- - - - -		-			- - - -	- - - - -		- -	- - - -	-		
	- - - - -	- - - -	-			- - - -	- - - - -		- -	- - - -	-		
	- - - - -	- - -	-			- - - -	- - - - -		- -	- - - -	-		
	- - -	- - -	-			- - - -	- - - - -		- -	- - - -	-		

+ = Fecal culture positive for Salmonella
- = Fecal culture negative for Salmonella

HB = Heart's blood culture

TABLE III. PRESENCE OR ABSENCE OF PROTEUS IN REPEATED CULTURES OF FECES

Irradiated Mice

Days before and after	Isolated in Individual Jars (24 21 18 14 11 9 5 1)	550 r x-radiation	Housed Together in Groups (3 7 10 14 17 21 24 26 31 35)
	+ + + + + + + + + + + -		- + + + + + + + + + ++ + + + + + D HB: Proteus
	- - - - - - - - - - - - - - - - - -		- - - - + D HB: Paracoli
	- - - - - - - - - - - - - - - - - -		- + - + + D HB: Proteus and Paracoli
	- - - - - - - - - - - - - - - - - -		- - - + + - + D HB: Proteus
	+ - - - - - - - - - - - - - - - - -		+ + + + - + - - - + D HB: Streptococcus
	- - - - - - - - - - - - - - - - - -		- - + + + + + + - + D HB: Coli and Paracoli
	+ - - - - - - - - - - - - - - - - -		+ - - + + + + + - + + + D HB: Paracoli
	+ + + + + + + + + + + -		- + + + + + D HB: Proteus
	- - - - + - - - - - - - - - - - - -		- - + - +D HB: Proteus and Coli
	+ - - - - - - - - - - -		- + + + + + + D HB: Proteus
	- - - - - + - - - - - -		- + + + + + + + + + + + + + + + + - + D HB: Proteus and Coli
	- - - - - - - - - - - - - - - - - -		- - + + + - - + + + + + - + + + + + + + + + + S
	+ - - - - - + - - - - - - - - - - -		- - + + + + + + + + + + + + + + - - + + + - - S

+ = Fecal culture positive for Proteus
- = Fecal culture negative for Proteus

HB = Heart's blood culture
S = Survived
D = Died

TABLE III. (Continued)

Unirradiated Mice

Days	Isolated in Jars						Housed in Groups																			Separated after Irradiation				
	16	13	10	6	5	1	1	5	8	12	15	19	22	25		1	4	6	10	14	
			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D	HB: Proteus		550 r x-radiation						
	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-			-	-	-	-	D	HB: Coli
	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	D HB: neg.
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-			-	-	-	-	-	S
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-			-	-	-	-	-	S
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	S
			-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	S
			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+	+	+	+	+	S
	+	-	-	-	-	-	-	-	+	+	-	+	+	-	-	-	-	-	-	D	HB: Coli, Paracoli									
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	KA	HB: neg.								
	+	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-			+	-	-	-	-	S
	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	S
	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-			-	-	-	-	-	S
	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	S

+ = Fecal culture positive for Proteus
- = Fecal culture negative for Proteus

HB = Heart's blood culture
KA = Killed and Autopsied
S = Survived
D = Died